

COMMONWEALTH



OF AUSTRALIA

Council for Scientific and Industrial Research

BULLETIN No. 220

LIBRARY
FISH & WILDLIFE SERVICE
PACIFIC OCEANIC FISHERY
INVESTIGATIONS

The Preparation and Use of Harvey's Reduced Strychnine Reagent in Oceanographical Chemistry

(Division of Fisheries—Report No. 13)

By

D. ROCHFORD, B.Sc.

MELBOURNE, 1947

Registered at the General Post Office, Melbourne, for transmission by post as a periodical.

Council for Scientific and Industrial Research

MEMBERS

Executive:

- Sir David Rivett, K.C.M.G., M.A., D.Sc., F.R.S.
(Chairman)
- A. E. V. Richardson, Esq., C.M.G., M.A., D.Sc.
(Chief Executive Officer),
- F. W. G. White, Esq., M.Sc., Ph.D.
- I. Clunies Ross, Esq., D.V.Sc.
- D. A. Mountjoy, Esq.

Chairmen of State Committees:

- A. J. Gibson, Esq., M.E.
(New South Wales)
- Professor E. J. Hartung, D.Sc.
(Victoria),
- R. J. Donaldson, Esq., D.S.O., B.C.E.
(Queensland),
- Professor Sir Kerr Grant, M.Sc.
(South Australia).
- P. H. Harper, Esq., B.A.
(Western Australia),
- F. H. Foster, Esq., B.C.E.
(Tasmania).

Co-opted Members:

- N. K. S. Brodribb, Esq., C.B.E., F.R.I.C.
- Sir Harry Brown, C.M.G., M.B.E.
- Professor W. J. Dakin, D.Sc.
- M. T. W. Eady, Esq.
- W. S. Kelly, Esq.
- E. H. B. Lefroy, Esq.
- G. Lightfoot, Esq., M.A.
- Professor Sir John Madsen, B.E., D.Sc.
- Professor H. C. Richards, D.Sc.
- J. P. Tivey, Esq., B.A., B.Sc., B.E.
- Professor S. M. Wadham, M.A., Dip. Agr.

Secretary:

G. A. Cook, M.C., M.Sc., B.M.E.

314 Albert Street,
East Melbourne,
Victoria.

COMMONWEALTH



OF AUSTRALIA

Council for Scientific and Industrial Research

BULLETIN No. 220

The Preparation and Use of Harvey's Reduced Strychnine Reagent in Oceanographical Chemistry

(Division of Fisheries—Report No. 13)

By

D. ROCHFORD, B.Sc.

MELBOURNE, 1947

Registered at the General Post Office, Melbourne, for transmission by post as a periodical

CONTENTS

	Page
Summary	5
1. INTRODUCTION	7
2. PREPARATION OF THE REAGENT	8
3. CALIBRATION OF THE REAGENT—	
(i) Method	9
(ii) Effect of HCl addition to the sample	11
(iii) Optimum dilution	16
(iv) Development time	16
(v) Working range	17
(vi) "Salt" error	18
(vii) Aging of the reagent	21
(viii) Comparative sensitivity of the reagent	21
(ix) Suggested method of calibration	23
4. LABORATORY USE OF THE REAGENT—	
(i) Visual Colorimeter	23
(ii) Photoelectric Absorptiometer	25
5. ACKNOWLEDGMENTS	27
6. BIBLIOGRAPHY	28
APPENDIX	29

SUMMARY

1. A method involving the use of a continuous atmosphere of CO_2 is given for the preparation of Harvey's reduced strychnine reagent.

2. It is demonstrated by a statistical analysis of the calibration data of a reagent prepared in this manner that:—

- (i) HCl addition to the sample improves its sensitivity.
- (ii) An optimum dilution of the stock reagent with sulphuric acid is characteristic of each reagent preparation.
- (iii) The optimum development time is below 1 hour.
- (iv) The working range of the reagent varies with the dilution.
- (v) Two determinations of the "salt" error of the reagent are given.
- (vi) The "aging" effect varies from batch to batch, but in general an increase in sensitivity takes place with time.
- (vii) By the use of a 0.0025 per cent. safranin reference solution, the reagent is shown to have a much greater sensitivity than that of Cooper's, prepared according to Harvey's method.
- (viii) A method involving statistical procedures for the calibration of the reagent is presented.

3. The laboratory procedure for the use of this reagent is detailed:—

- (i) Using a visual colorimeter.
- (ii) Using a photoelectric absorptiometer.

The Preparation and Use of Harvey's Reduced Strychnine Reagent in Oceanographical Chemistry*

By D. Rochford, B.Sc.

1. Introduction

For the determination of nitrate nitrogen in oceanographical chemistry, one reagent is that of Denigès (1911), as modified for use with seawater by Harvey (1926, 1928*a*, 1928*b*) and others.

Some criticism (Thompson and Johnson, 1930) has been levelled against the use of this reagent, but in the author's opinion many of its defects can be attributed to faulty preparation of the reduced strychnine compound, and its incorrect dilution with sulphuric acid.

In the method of preparation given by Harvey (1928*b*), the strychnine salt is reduced in the beginning of the process by nascent hydrogen liberated from the zinc hydrochloric acid reaction, but thereafter the reducing atmosphere is maintained by the slow evolution of hydrogen from the zinc mercury couple. It is during the latter period that the greater part of the concentrating of the reduced strychnine solution, consisting mainly of strychnidine and tetrahydro strychnine (Zwicker and Robinson, 1944), to the final volume of some 125 ml., takes place.

The apparatus used by Harvey (1928*b*), could only imperfectly shield the reduction products from atmospheric oxidation, during this concentrating phase, and it is probable that the olive-green product thus obtained is a mixture of the required reduction products and some re-oxidized material. In a communication to Wattenberg, Rakestraw (Wattenberg, 1937), states that the reduced strychnine solution should be colourless. Rakestraw also observed that the reduced strychnine is very sensitive to atmospheric oxidation, and suggested that the addition of the sulphuric acid to the reduced strychnine should be done in an inert atmosphere. In recent papers Zwicker and Robinson (1942, 1944) used pure strychnidine dissolved in sulphuric acid as a reagent for the determination of nitrates. This reagent is colourless.

Cooper (1932), following Harvey's original method of preparation, has experienced little difficulty in obtaining good batches of reagent from the majority of the reduced strychnine solutions. Riddell (1936), also uses Harvey's method without difficulty, but devoted some time to an investigation of the use of the reagent.

Following upon some earlier difficulties in the preparation of the reagent, a method was developed in this Laboratory for the preparation of the reduced strychnine, and subsequent acidification with sulphuric acid in a continuous atmosphere of carbon dioxide gas. Details of this method of preparation, and of the subsequent calibration and use of this reagent, are given in this paper.

* Typescript received May 27, 1946.

2. Preparation of the Reagent

The quantities of strychnine sulphate, hydrochloric acid, and distilled water are the same as given by Harvey (1928b). Care must be taken in the selection of the hydrochloric acid, and only the highest grade analytical reagent should be used. However, it has been found that 100 g. of zinc amalgam is sufficient for the reduction. A diagram of the apparatus in which the preparation is carried out, is given in Fig. 1.

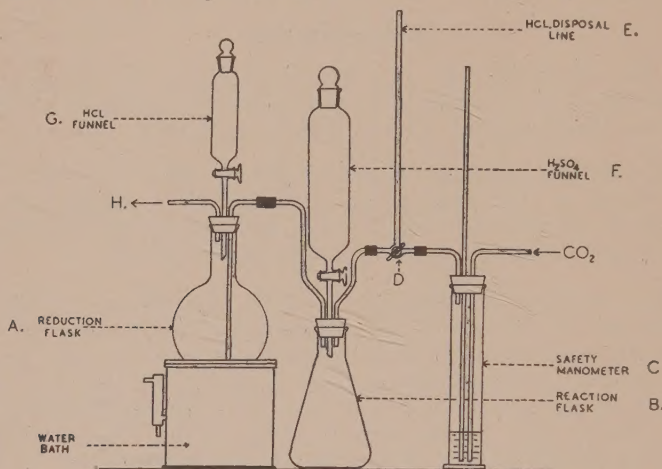


FIG. 1.—Diagram of apparatus for the preparation of Harvey's reagent.

The strychnine sulphate, zinc amalgam, and distilled water are placed in flask A, and heated over the water bath. Carbon dioxide is passed through the apparatus for several minutes at the rate of about 10 drops per second via the bubbler C, to displace atmospheric gases. The hydrochloric acid is then added from G in 25-ml. lots, at about 15-minute intervals, the flow of carbon dioxide being maintained at the same rate. When the vigorous zinc-hydrochloric acid reaction has been completed, the carbon dioxide flow can be increased.

Assisted by the flow of carbon dioxide through A, the concentrating of the reduction product to a volume of 100-125 ml. is accomplished in 15-20 hours. The reduced strychnine solution thus obtained is colourless. When the required volume has been reached, the carbon dioxide supply is connected to flask A at H, the two-way tap at D adjusted so that the connection to E is established, and a sufficient pressure of carbon dioxide built up in A to drive the solution over into flask B, and maintain a slow circulation of carbon dioxide through the system.

The sulphuric acid which can be freed from any traces of nitrates by the ammonium sulphate method described by Wattenberg (1937), is now added slowly and with shaking of the reaction flask, from F, until 500 ml. of the acid has been introduced.

The hydrogen chloride gas generated during this addition is swept out at E by the carbon dioxide gas stream, and can be conducted by glass tubing to any convenient disposal point. The solution in the reaction flask should be allowed to stand in a carbon dioxide atmosphere for about a day until all the zinc sulphate has settled and the supernatant liquid then decanted off into dark glass bottles. This is called the *stock reagent*.

Harvey (1928*b*) considers that the reagent should be allowed to stand about six weeks before use. A reagent prepared in the above manner will give satisfactory results even within several days of preparation. The calibration data given in the subsequent sections of the paper were obtained with reagents less than one week old.

Harvey (1928*b*) dilutes each preparation of the reduced strychnine with 1 litre of sulphuric acid, but in the author's opinion the reagent is less subject to subsequent deterioration if kept in a lower acid concentration, generally in the ratio of 500-700 ml. of acid to 125-150 ml. of the reduced strychnine.

3. Calibration of the Reagent

(i) *Method.*

The stock reagent was diluted with nitrate-free H_2SO_4 so as to give a series of dilutions ranging up to a twelvefold, or even greater dilution. Since June 1945, dilutions of one, three, six, and twelvefold have been adopted as standard practice.

The following standard nitrate solutions were prepared by appropriate dilution of the stock nitrate standard of 2,500 μg . nitrate nitrogen per litre:—

0, 25, 50, 75, 150, 250, 500 μg . N per litre. Prior to June 1945, two of these standard series were prepared for each dilution of the stock reagent. One was treated with two drops of HCl, and the other left untreated before the addition of the nitrate reagent. As will be shown later, this addition of HCl greatly improves the efficiency of this reagent, and its addition has been adopted as standard practice since June 1945.

It has been found most convenient to develop the colour of the reagent-nitrate standard mixture in 30 ml. Erlenmeyer flasks which can be stored in a lightproof box. 5 ml. of each of the standard solutions are placed in order in these flasks in the developing box, 2 drops of HCl added, followed by 5 ml. of the reagent. The reagent is run into the bottom of the flask and allowed to remain as a bottom layer until all the reagent additions have been made. Then the flasks are shaken in order and the time noted. At standard intervals of $\frac{1}{4}$ to $\frac{1}{2}$ hour, 1 to 2 hours, 20 to 25 hours, the successive balance points of the standards against one of the series (generally the 75 μg . N per litre standard) are obtained with a visual colorimeter or their adsorption relative to that of a distilled water blank ascertained with an absorptiometer (see page 25). Finally, one of the standards is balanced against a solution of 0.0025 per cent. safranin. This safranin solution is arbitrarily assigned the value of 100 colour units. By this means the safranin colour equivalents of these standard nitrate solutions for each dilution of a particular batch of reagent at various development times, with and without HCl addition, have been ascertained.

The calibration data for the reagent preparations from August 1944 to January 1946 are given in the Appendix.

It is necessary to point out that all these calibration data have been obtained with standard nitrate solution, made up in distilled water. The effect of seawater upon the calibration data of Harvey's reagent will be

given in Section 3 (v). From these calibration data by the method of least squares, the regression lines for each batch of reagent at the various dilutions and development times, with and without HCl addition*, were calculated. For convenience in analysis the original nitrate values have been divided by 25 prior to the calculation of these regression lines.

The equation is in the linear form $Y = bx \pm a$ where Y represents safranin colour unit and x concentration of a nitrate solution (standard). The constant b of this equation under these circumstances is a measure of the sensitivity of the reagent, being related to θ the angle of intercept by the equation $\tan \theta = b$ (see Fig. 2). The sensitivity of colour

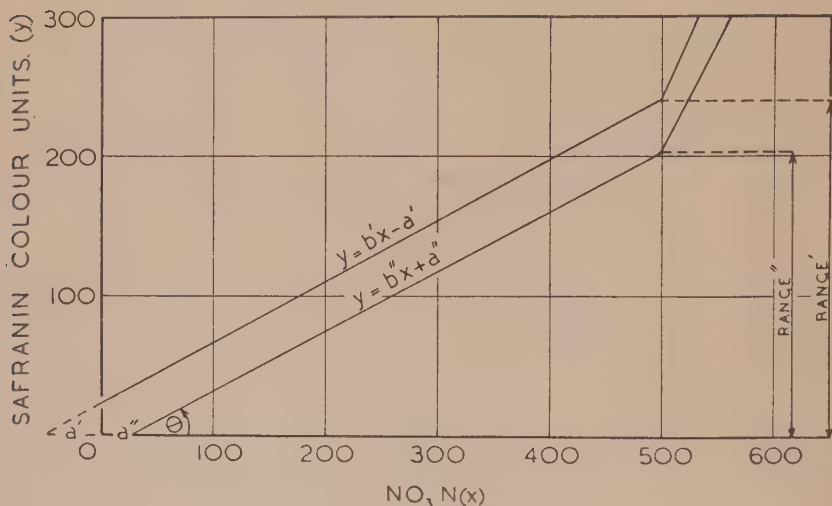


FIG. 2.—Explanatory calibration graph.

development per unit of nitrate nitrogen varies directly with θ so that the greater the value of b the greater is the sensitivity.

The constant a is a measure of the blank value of the reagent and should lie within certain specified limits and preferably have a negative value.

The values $Sy.x$ in relation to each regression coefficient are standard errors of estimate of these coefficients and may be used in the usual manner. Estimates of the significance of the regression coefficients given as t values indicate the usefulness of the various regression lines.

The "range" is considered to be the upper limit of nitrate nitrogen concentration beyond which no significant change in colour development takes place. This has been termed the asymptotic level, and is generally discernible only with the higher dilution of the reagent; cf. Figs. 2 and 10. In Table 1 the statistical data for the April 1945 reagent are given as an example of the type and range of data obtained by this method of analysis.

* Prior to June 1945, cf. p. 11, sub-section (ii).

From a consideration of these statistical values, it is possible to select the dilution and time of development at which the reagent possesses the following characteristics:—

- (a) Greatest sensitivity, i.e., greatest value of b consistent with permissible limits of $Sy.x$ and t values.
- (b) Least blank error, i.e., smallest value of a .
- (c) Working range sufficient to cover normal requirements.

In the subsequent Sections 3 (ii)—3 (vii), consideration is given to the effects of certain variables upon these desiderata.

TABLE 1.—STATISTICAL ANALYSIS OF THE CALIBRATION DATA OF THE APRIL 1945 REAGENT.

HCl.	Dilution.	Time.	a .	b .	$Sy.x$.	t .	Range.
		hr.					$\mu\text{g/l.}$
+	$\times 1$	$\frac{1}{2}$	— 4.7	8.5	74	2	500
—	$\times 1$	$\frac{1}{2}$	— 1.5	2.2	36	1.06	500
+	$\times 1$	$1\frac{1}{2}$	— 7.5	11.3	56	3.51	500
—	$\times 1$	$1\frac{1}{2}$	— 3.1	3.8	24	2.75	500
+	$\times 1$	18	9.7	7.9	171	0.745	500
—	$\times 1$	18	2.5	4.3	26.7	2.9	500
+	$\times 3$	$\frac{1}{2}$	— 6.4	15.7	65	4.2	500
—	$\times 3$	$\frac{1}{2}$	— 2.2	4.6	7	11.44	500
+	$\times 3$	18	4.8	13.8	217	1.1	500
—	$\times 3$	18	— 1.7	8.2	32	4.55	500
+	$\times 6$	$\frac{1}{2}$	— 1.6	19.1	71	4.68	500
—	$\times 6$	$\frac{1}{2}$	— 1.2	6.5	13	4.3	500
+	$\times 6$	22	9.4	19.4	49	6.89	500
—	$\times 6$	22	— 2.8	12.5	90	2.41	500
+	$\times 12$	$\frac{1}{2}$	15	13.3	240	0.76	500
—	$\times 12$	$\frac{1}{2}$	4	3.5	15	1.94	250
+	$\times 12$	22	7.31	13.7	38	3.04	250
—	$\times 12$	22	1.9	7.3	36	3.52	500

(ii) Effect of HCl Addition to the Sample.

Riddell (1936) was the first to use hydrochloric acid with Harvey's reagent. He found that the addition of hydrochloric acid to the samples prior to the addition of the strychnine reagent greatly increased the sensitivity of the reagent. The plottings of the b values derived from the calibration data of the August 1944, November 1944, the two batches of February 1945, and the April 1945 reagents (Figs. 3 to 7), show that the prior addition of HCl in most cases increases the sensitivity of the reagent at all dilutions and especially at the lesser development times.

Sometimes, at the end of 20-24 hours development, the sensitivity with HCl addition is equal to, or slightly less, than that without HCl addition. The effect is shown in Figs. 5 and 6 at 15-20 hours development. In the case of the April 1945 reagent (Fig. 8), HCl addition greatly

increases the sensitivity at all dilutions and development times. It is suggested therefore that the prior addition of HCl as originally proposed by Riddell (1936) does increase the sensitivity of Harvey's reagent, and is to be recommended for general use.

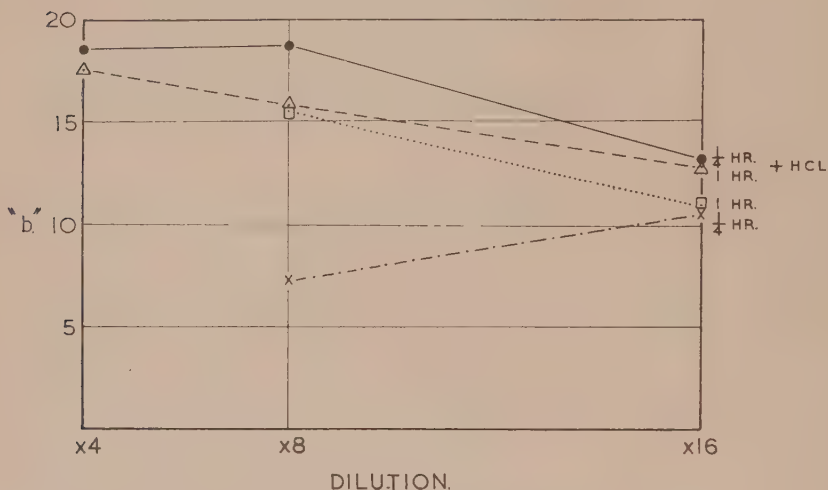


FIG. 3.—The variation of sensitivity with dilution of the August 1944 reagent.

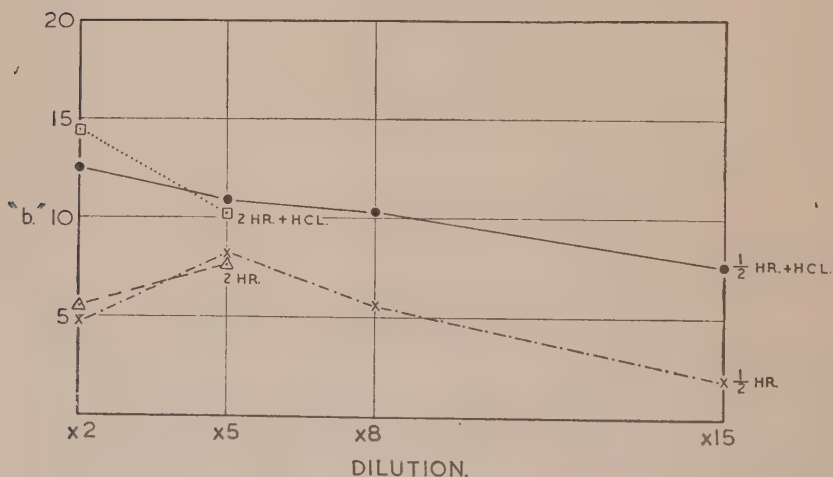
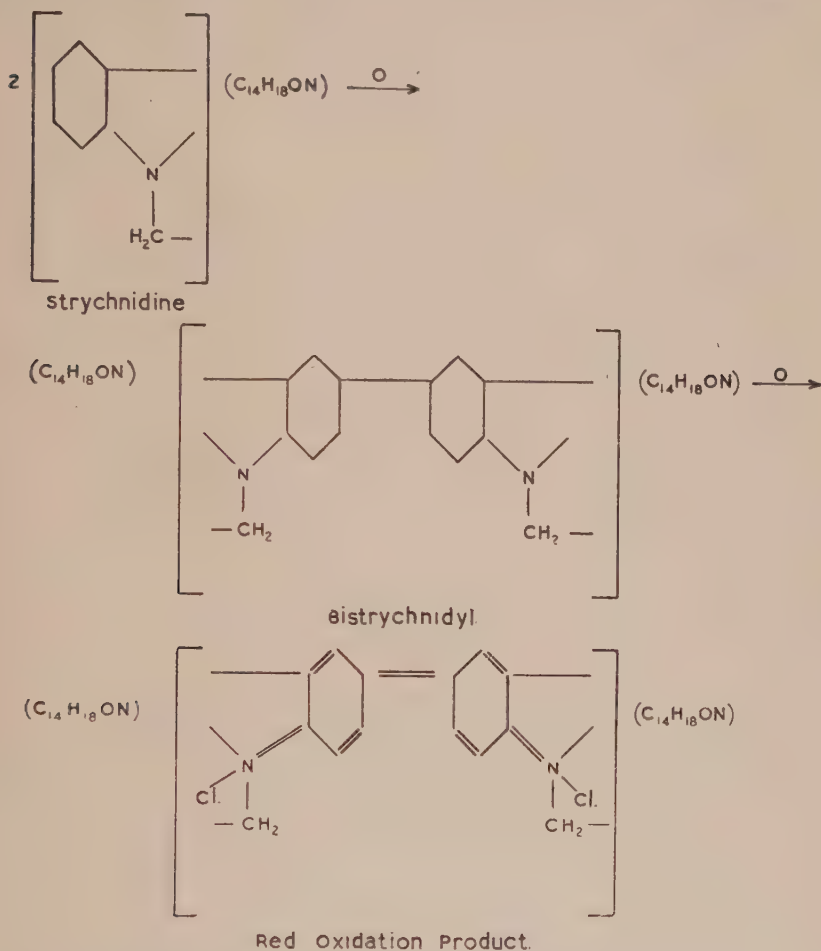


FIG. 4.—The variation of sensitivity with dilution of the November 1944 reagent.

In the Hydrological Laboratory of the Fisheries Division, it has been standard practice since June 1945 to add 2 drops of HCl to the samples and standards before the addition of the reagent.

According to Zwicker and Robinson (1944) the equation for the oxidation of strychnine to the final red oxidation compound is as follows:—



It is noteworthy that the final oxidation product contains two chlorinated nitrogen groups. It is suggested that the hydrochloric acid provides a readily available source of chlorine for the formation of these groups, which probably intensify the colour strength of the final oxidation compound. Conversely, the varying amounts of the hydrochloric acid normally present in the reagent provide a variable source of chlorine which becomes available more slowly for the formation of these chlorinated nitrogen groups. The colour-intensifying effect of HCl addition would thus be most apparent at the lesser development times.

It is suggested that the almost complete coincidence of the sensitivity curves for the February 1945 reagents (Figs. 5 and 6), at the end of 15-20 hours development, can be explained on the basis of these reagents having sufficient HCl of composition to chlorinate the nitrogen groups of the oxidized strychnine compounds at these longer development times, and thus develop maximum colour intensities.

In the case of the April 1945 reagent (Fig. 7), it is suggested that the reagent was prepared with a relative deficiency of HCl, and therefore even at the end of 24 hours development a relative deficiency of the oxidation compounds containing the chlorinated nitrogen group prevented the development of maximum colour intensity.

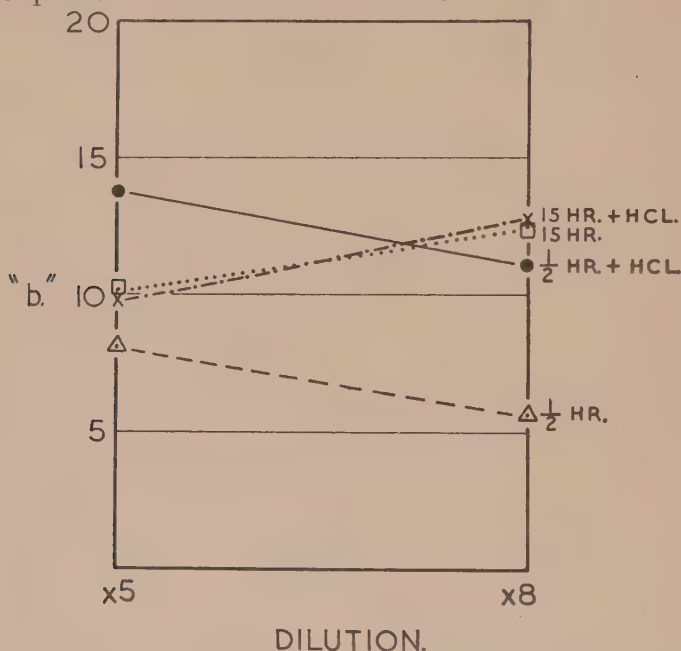


FIG. 5.—The variation of sensitivity with dilution of the February 1945a reagent.

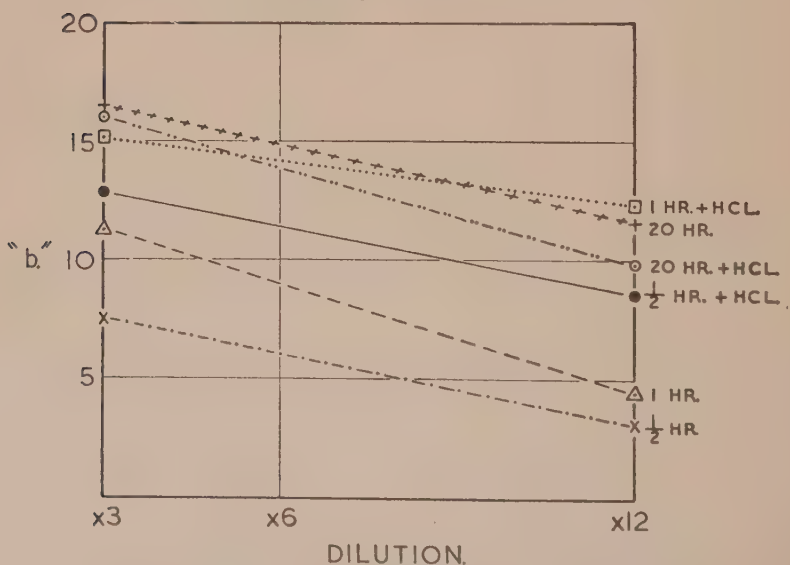


FIG. 6.—The variation of sensitivity with dilution of February 1945b reagent.

A reagent prepared in this laboratory, under reduced pressure, was almost free of hydrochloric acid. The reagent had a very low sensitivity which was greatly increased by saturation of the reagent with hydrochloric acid, and this is considered as further evidence of the importance of HCl for the most efficient functioning of the reagent.

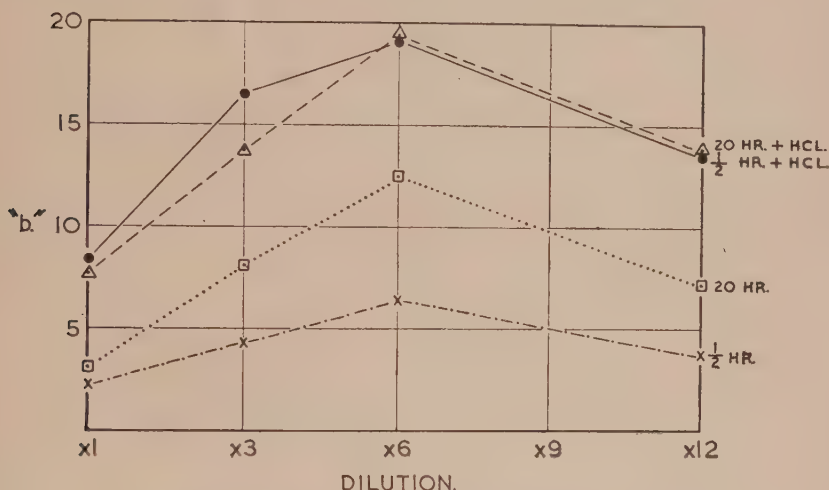


FIG. 7.—The variation of sensitivity with dilution of the April 1945 reagent.

In Fig. 8 the value of the constant a from the regression lines of the April 1945 reagent has been plotted as a function of dilution and development times. It can be seen that the addition of HCl does increase the blank value of the reagent, but only at the longer development does this effect become significant. At the optimum dilution, which was selected as sixfold for this reagent (Table 5), the blank value at the end of $\frac{1}{2}$ hour development was — 1.6 safranin units, which is the equivalent of about $3\mu\text{g.}$ nitrate nitrogen per litre.

In Figs. 13 and 15, the a values of the December 1945 and January 1946 reagents are plotted as a function of time and dilution. With the December 1945 reagent (Fig. 13), there is a blank value of 5 safranin or ca. 7.5 nitrate nitrogen units at the optimum dilution of threefold and $\frac{1}{2}$ hour development. At greater dilutions and longer development times the blank values are such as to invalidate the use of the reagent at nitrate concentrations below 25 $\mu\text{g.}$ nitrate nitrogen per litre.

The January 1946 reagent (Fig. 15) has a blank value of 7.5 safranin, or 15 nitrate nitrogen units at the optimum dilution of sixfold and $\frac{1}{2}$ hour development. There does not appear to be any systematic variation in the a value of this reagent with dilutions or development.

It is the author's experience that the blank value of the strychnine reagent is largely governed by the purity of the hydrochloric acid used in its preparation, and subsequent acidification of the sample. Care must be exercised therefore in the selection of the hydrochloric acid used in the preparation of the reagent.

(iii) *Optimum Dilution.*

The optimum dilution or that dilution of the original stock reagent which gives a reagent with the characteristics conforming to certain specifications in regard to sensitivity, reagent blank, and working range, varies from preparation to preparation.

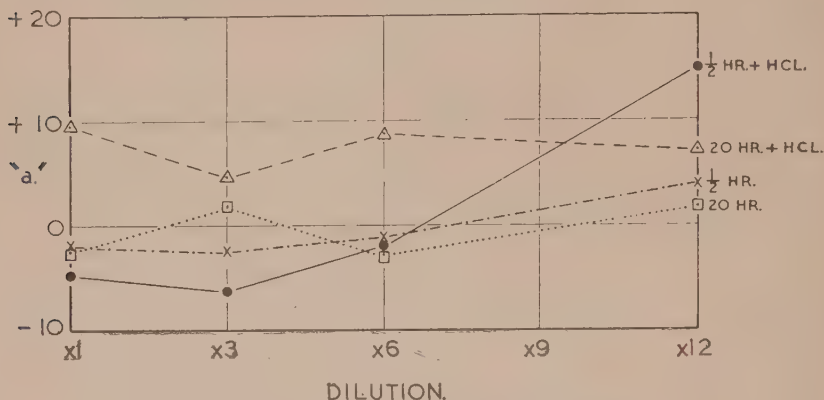


FIG. 8.—The variation of the blank constant with dilution of the April 1945 reagent.

The effect of dilution upon the sensitivity of the reagent is very clearly demonstrated in Figs. 3 to 14, where the b constants are plotted as functions of time and dilution.

Generally there appears to be a big increase in sensitivity at the threefold compared with the undiluted stock reagent, and this increase demonstrates the importance of this optimum dilution for the most efficient functioning of each reagent preparation.

Between a three and sixfold dilution there is often little difference in sensitivity, but at a twelvefold dilution the sensitivity has generally decreased to a value approaching that of the undiluted reagent. Moreover, at the optimum dilution little increase in sensitivity takes place with longer colour development, cf. Figs. 11, 12, and 14.

(iv) *Development Time.*

Harvey (1928a) considers that the reagent should be allowed to react for 24 hours to obtain maximum colour development.

The plottings of the sensitivity constants b as a function of dilution and time (Figs. 3 to 14) demonstrates that with reagents prepared in the manner indicated in Section 2, sensitivities equal or better than those obtained at 24 hours development are obtained at $\frac{1}{2}$ to 1 hour development. If, besides sensitivity, other desirable features such as reagent blank and variance are taken into consideration, the results obtained with $\frac{1}{2}$ –1 hour development are generally superior to those at 20–24 hours.

From a consideration of the calibration data of these reagents at their optimum dilution, it is apparent that the minimum values of a are, for the most part, obtained at $\frac{1}{2}$ –1 hour development. Moreover, the

$Sy.x$ and t values of these reagents at their optimum dilution show that the variance of the calibration data about the regression line, i.e., its degree of conformity to Beer's law, is generally at a minimum for the lower development time.

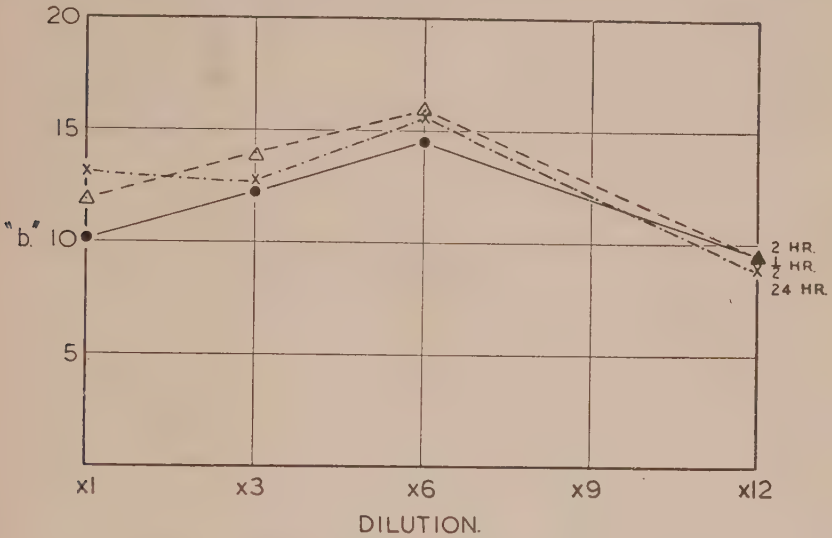


FIG. 9.—The variation of sensitivity with dilution of the May 1945 reagent.

(v) Working Range.

In Fig. 10 are plotted the calibration data for the May 1945 reagent at a dilution of one and eighteenfold, and $\frac{1}{2}$, 2, 24 hours development.

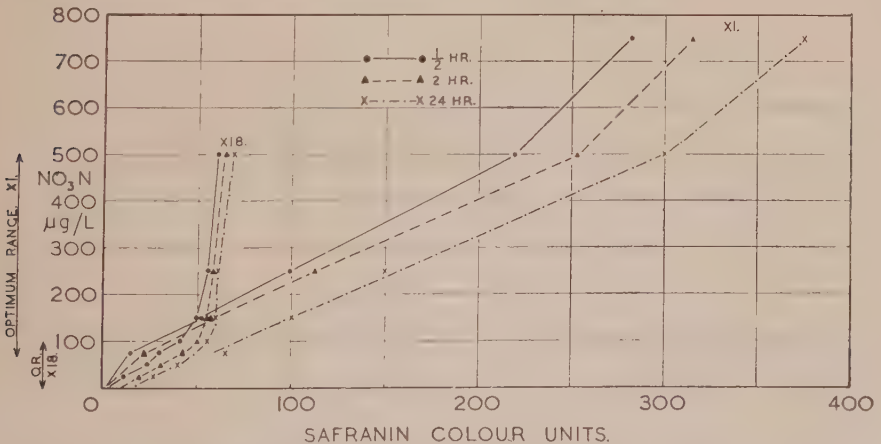


FIG. 10.—Calibration curves of the May 1945 reagent at dilutions of one and eighteenfold.

From an inspection of the plotted data it is evident that Beer's law does not apply over the whole range of nitrate values examined. At the eighteenfold dilution, Beer's law appears valid only up to a nitrate

concentration of 100 $\mu\text{g. N}$ per litre. At the onefold dilution, Beer's law appears to apply over the range 75–500 $\mu\text{g. N}$ per litre. At the onefold dilution there is a better approximation to Beer's law over the range 0–750 $\mu\text{g. N}$ per litre at 24 hours development. With the eighteen-fold dilution, the converse appears to apply.

In Table 2 are given the b and a constants of the regression line, derived from the calibration data covering the working ranges appropriate to the dilution, and for comparison the regression constants derived from the calibration data over the whole range of nitrate concentrations. The $Sy.x$ and t figures are also given in this table.

TABLE 2.—STATISTICAL ANALYSIS OF THE CALIBRATION DATA OF THE MAY 1945 REAGENT IN RELATION TO "WORKING RANGE."

HCl.	Dilution.	Time.	a .	b .	Sy	t .	Range.
		hr.					$\mu\text{g.}$
+	$\times$ 1	$\frac{1}{2}$	— 1.9	11.2	385	0.093	0 — 750
+	$\times$ 1	$\frac{1}{2}$	— 20.8	12	15.5	10	75 — 500
+	$\times$ 1	2	— 2.0	11.2	381.8	0.746	0 — 750
+	$\times$ 1	2	31.5	13.3	20	9.7	75 — 500
+	$\times$ 1	24	22.6	12.5	448	0.089	0 — 750
+	$\times$ 1	24	17.6	13.9	68.5	2.6	75 — 500
+	$\times$ 18	$\frac{1}{2}$	19.3	2.6	162	0.035	0 — 750
+	$\times$ 18	$\frac{1}{2}$	2.6	9.8	1.7	19.6	0 — 100
+	$\times$ 18	2	26.4	2.4	177.5	0.235	0 — 750
+	$\times$ 18	2	7.8	11.0	3.7	9.2	0 — 100
+	$\times$ 18	24	33.7	2.2	175	0.027	0 — 750
+	$\times$ 18	24	14.8	11.1	10.7	3.4	0 — 100

From a consideration of this table it is apparent that at an eighteen-fold dilution Beer's law is closely followed up to 100 $\mu\text{g. N}$ per litre, the sensitivity over this working range being little different from that at a onefold dilution. Moreover, the working range appears to diminish with longer development. At the onefold dilution Beer's law is followed very closely over the 75–500 $\mu\text{g. N}$ per litre working range. However, at this dilution the working range appears to increase with development time, so that at the end of 24 hours, Beer's law is followed between 0 and 500 $\mu\text{g. N}$ per litre.

These data demonstrate that the working range of a reagent is a function of its dilution, and consideration must be given to the required working range when selecting the optimum characteristics of a reagent.

(vi) "Salt" Error.

Harvey (1928*b*), in the original publication dealing with the use of this reagent, states that it is subject to a "salt" error when used with sea-water samples. He suggested the addition of sodium chloride to the standard nitrate solution to counteract this effect. Since that date a number of workers (Thompson and Gilson, 1937, Thomsen, 1937) have prepared the comparison nitrate standards by dilution of the stock nitrate

solution with nitrate-free seawater of the appropriate chlorinity. In this laboratory large numbers of both oceanic and estuarine samples are analysed for nitrates, and chlorinities ranging from those of normal seawater down to freshwater are encountered. It has been considered inconvenient and time consuming to make up separate nitrate standards for each chlorinity and all standards are consequently made up in distilled water.

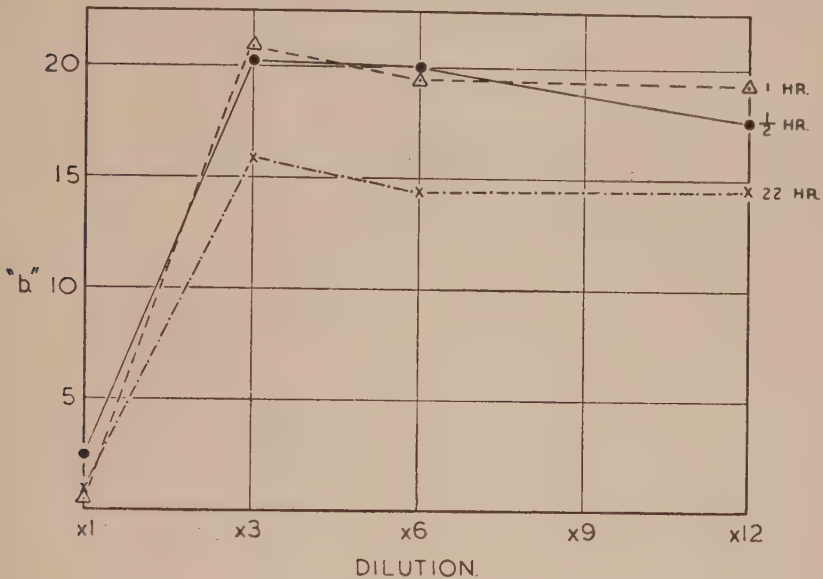


FIG. 11.—The variation of sensitivity with dilution of the June 1945 reagent.

Two determinations of the "salt" error of Harvey's reagent were made with the February 1945*b* reagent. The calibration data up to 500 $\mu\text{g. N}$ per litre were obtained, as in Section 3 (i), with standards made up in distilled water, and in seawater containing 9.38–9.50 per mille Cl, and 19.50–19.65 per mille Cl. The reagent was used at a threefold dilution and the colour developed for one hour. The first determination of "salt" error was made on February 22, 1945, and the second on March 20, 1945.

In Table 3 are given the regression constants, and the $Sy.x$ and t values calculated from the calibration data of these two "salt" error determinations. The salt error from each chlorinity is the numerical factor necessary to adjust the slope of the regression line (at that chlorinity) to that of the regression line obtained from the distilled water calibration data. In Table 4, the values of these correction factors are entered.

The effect of increasing chlorinity upon the blank values obtained at each determination is noteworthy, as also the increase in the $Sy.x$ deviation with increasing chlorinities.

TABLE 3.—STATISTICAL ANALYSIS OF THE CALIBRATION DATA OF THE FEBRUARY 1945 REAGENT AT VARYING CHLORINITIES.

Date.	Reagent.	HCl.	Dilu- tion.	Time.	Cl per Mille.	<i>a.</i>	<i>b.</i>	<i>Sy.x.</i>	<i>t.</i>	Range.
				hr.						$\mu\text{g.}$
Feb. 22 1945	Feb. 1945b.	+	$\times 3$	1	19.5	3.1	10.1	79.8	2.1	0 — 500
	Feb. 1945b.	+	$\times 3$	1	9.5	0.2	11.2	44.8	4.1	0 — 500
	Feb. 1945b.	+	$\times 3$	1	aq. . dist.	-2.8	15.1	31.2	8.4	0 — 500
Mar. 20 1945	Feb. 1945	+	$\times 3$	1	19.65	6.9	11.6	137.5	1.3	0 — 500
	Feb. 1945	..	$\times 3$	1	9.38	-1.6	12.9	60.8	3.4	0 — 500
	Feb. 1945	+	$\times 3$	1	aq. dist.	-5	16.6	40	6.0	0 — 500

TABLE 4.—“SALT” ERROR OF THE FEBRUARY 1945 REAGENT AT VARYING CHLORINITIES.

Date.			Correction Factor.	
			9.5 Cl Per Mille.	19.6 Cl Per Mille.
22nd February, 1945	..	..	1.35	1.49
20th March, 1945	..	..	1.29	1.43
Mean	..	..	1.32	1.46

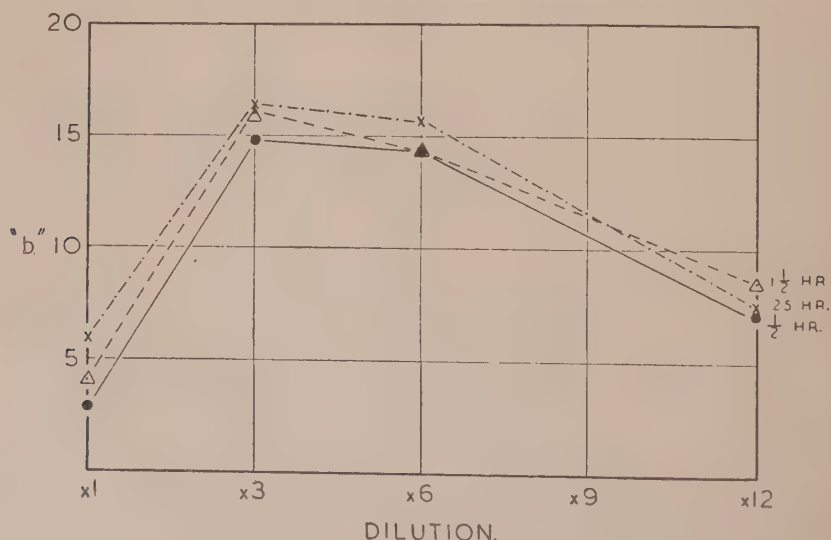


FIG. 12.—The variation of sensitivity with dilution of the December 1945 reagent.

Riddell (1936) has found a salt inhibitory factor of 1.25 at a salinity of 30–35 per thousand, and Wattenberg, in a report to Sund (1929) states that a salt error becomes apparent above the salinity of 8 per thousand.

(vii) "Aging" of the Reagent.

Harvey (1928a) allowed his preparation to stand about a week before use, and it is suggested by Zwicker and Robinson (1944) that during this time a portion of the tetrahydro-strychnine is converted to the more nitrate-sensitive strychnidine.

With a reagent prepared as in Section 2, sometimes little change in the sensitivity characteristics takes place with age. With the May 1944 reagent (Fig. 16), the effect of age has been to diminish the working range of the reagent, and this appears to be a common occurrence. In Fig. 17, plottings of the standard curve of the November 1944 reagent under optimum conditions at an interval of about five months are given. It can be seen that very little change in the slope of the standard curve has taken place in this time interval. With the February 1945 reagent, a much more sensitive reagent developed at the end of one month's aging (Table 3).

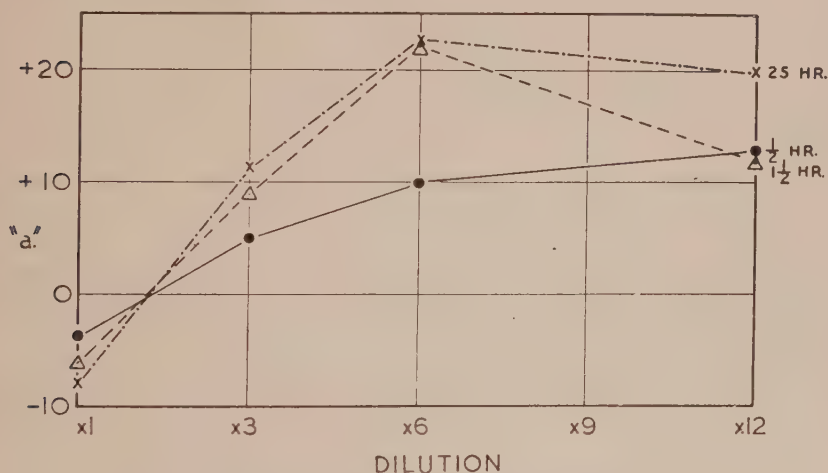


FIG. 13.—The variation of the blank constant with dilution of the December 1945 reagent.

It would appear that a mixture of tetrahydro-strychnine and strychnidine will, if a definite partition ratio is not initially present, adjust itself in time to this ratio. Under these conditions, the aging effect would be most pronounced with these reagents, having an initially high relative concentration of tetrahydro-strychnidine (assuming this latter compound to be the least stable). As the relative proportion of tetrahydro-strychnine probably varies from preparation to preparation the calibration characteristics of a reagent must be checked at intervals during use.

(viii) Comparative Sensitivity of the Reagent.

In order to make comparison of reduced strychnine reagents possible on an absolute basis, Cooper (1932) suggested the use of a solution of 0.0008 per cent. safranin. He considered that with a good batch of the

reagent a colour corresponding to that of this solution should be developed with a nitrate standard solution containing 90–130 $\mu\text{g. N}$ per litre.

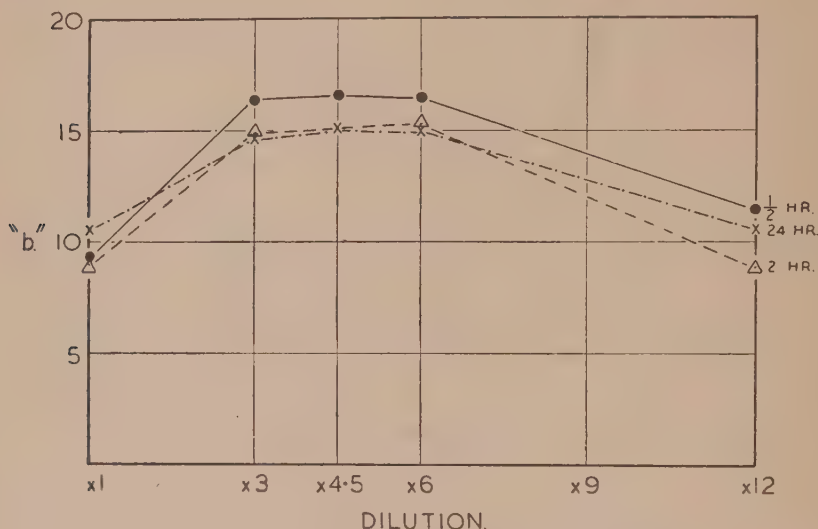


FIG. 14.—The variation of sensitivity with dilution of the January 1946 reagent.

In this laboratory a 0.0025 per cent. safranin solution has been used as a reference solution and an equivalent colour is developed with a nitrate solution lying between 100 to 200 $\mu\text{g. nitrate nitrogen}$ per litre.

It would thus appear that a reagent prepared in the manner indicated in Section 2 will have a much greater sensitivity than one prepared according to Harvey's (1928*b*) method. This greater sensitivity, it is suggested, is brought about by the fuller elimination of any reoxidized material in the reagent.

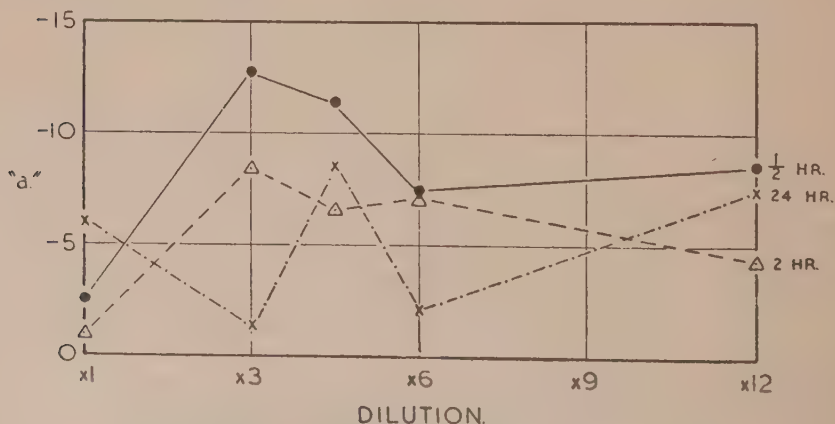


FIG. 15.—The variation of the blank constant with dilution of the January 1946 reagent.

(ix) *Suggested Method of Calibration.*

From the data presented in this publication it is evident that the characteristics of the nitrate reagent vary from preparation to preparation. However, provided a method of preparation which will minimize the amount of reoxidation products in the reagent is followed, a minimum of variation in these characteristics will be found. It is felt that the preparation of the reagent in an atmosphere of CO_2 ensures a minimum content of reoxidized material in the reagent.

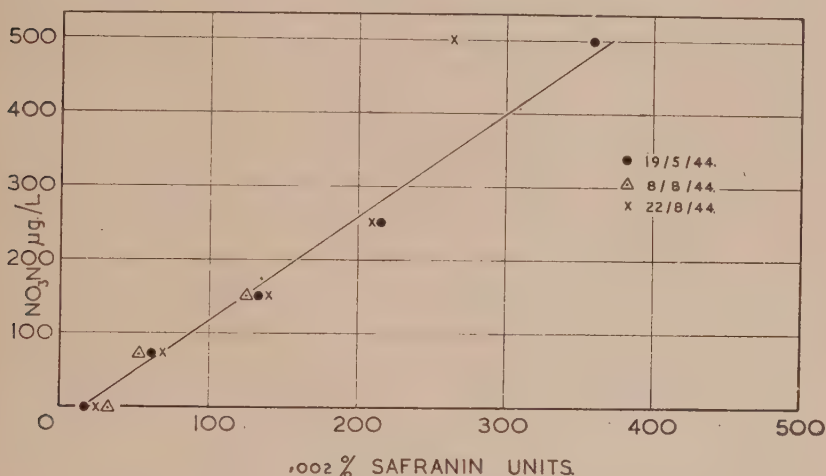


FIG. 16.—“Aging” effect upon the standard curve of the May 1944 reagent.

In this laboratory it is now the standard practice to ascertain, as in Section 3 (i), the calibration data of a new reagent preparation in terms of safranin colour units at a one, three, six, and twelvefold dilution. Development times of $\frac{1}{2}$, 2, and 24 hours have been adopted. The regression constants, plus the $Sy.x$ and t values, are calculated and that dilution and development time selected at which the reagent conforms to the specification given in Table 5.

The optimum dilutions and development times of the August 1944 to January 1946 reagents, selected on this basis from a consideration of their calibration characteristics, are given in Table 5.

4. Laboratory Use of the Reagent

(i) *Visual Colorimeter.*

Once the optimum characteristics of a reagent have been ascertained it is necessary to construct a standard curve for its laboratory use. This is prepared from the calibration data by assigning 100 arbitrary colour units to the 75 $\mu\text{g. N}$ per litre standard, calculating the other standards in terms of these colour units, and constructing a graph relating colour intensity to nitrate concentration.

In Fig. 18 the standard curves of the August 1944 reagent are given. It is convenient to plot the standard curves in the two ranges, 0–150 and 0–500 $\mu\text{g. nitrate nitrogen per litre}$.

TABLE 5.—OPTIMUM CHARACTERISTICS OF THE REAGENT PREPARATIONS FROM AUGUST 1944 TO JANUARY 1946.

Reagent.	HCl.	Dilution.	Time.	<i>a.</i>	<i>b.</i>	<i>Sy.x.</i>	<i>t.</i>	Range.
			hr.					$\mu\text{g.}$
August, 1944 ..	+	$\times 4$	$\frac{1}{4}$	10.3	18.6	168	1.9	0 — 500
November, 1944 ..	+	$\times 2$	$\frac{1}{2}$	11.4	12.6	56.8	3.7	0 — 500
February, 1945a.	+	$\times 5$	$\frac{1}{5}$	— 2.2	13.8	11	21.2	0 — 250
February, 1945b.	+	$\times 3$	1	2.8	15.1	31.2	8.4	0 — 500
April, 1945 ..	+	$\times 6$	$\frac{1}{6}$	— 1.6	19.1	71	4.68	0 — 500
May, 1945 ..	+	$\times 6$	$\frac{1}{6}$	— 3.2	14.6	8.28	15.16	0 — 250
June, 1945 ..	+	$\times 3$	$\frac{1}{3}$	0.9	20.3	157	2.23	0 — 500
December, 1945 ..	+	$\times 3$	$\frac{1}{3}$	5	14.9	24.2	10.6	0 — 500
January, 1946 ..	+	$\times 6$	$\frac{1}{6}$	— 7.4	16.4	84.7	3.3	0 — 500
Maximum ..	..	..	..	11.4	20.3	168	21.2	
Minimum ..	..	..	..	— 7.4	12.6	8.3	2.23	
Suggested Specifications ..				0 ± 10	>12.5	<150	>2	

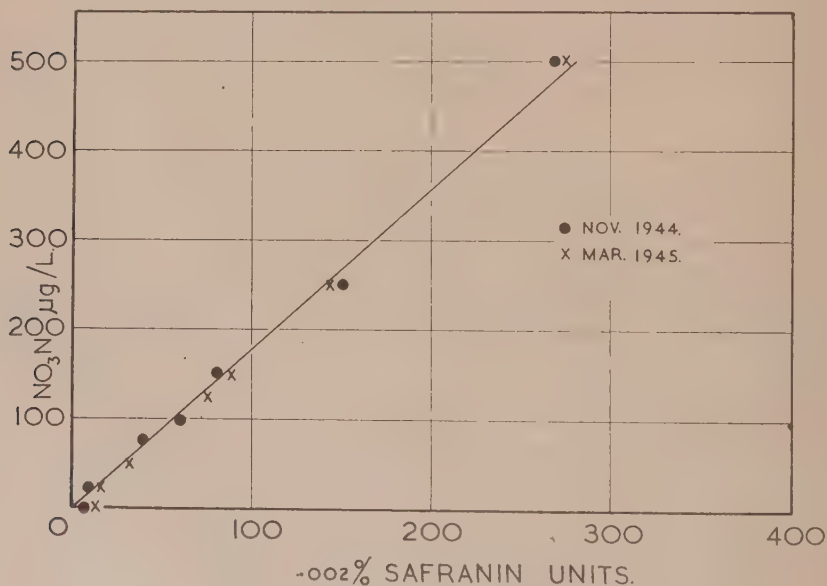


FIG. 17.—“Aging” effect upon the standard curve of the November 1944 reagent.

For each batch of nitrate determinations it is sufficient to use a 75 $\mu\text{g. N}$ per litre solution as standard. 5 ml. of this standard and of each of the samples are added to 30-ml. Erlenmeyer flasks; 2 drops of HCl are added to each flask, and sufficient of the stock reagent diluted to its optimum dilution; 5 ml. of the diluted reagent is added to each flask in turn and allowed to remain as a bottom layer until all additions have been made. Each flask is then shaken in turn, replaced in the

developing box (Fig. 20), and allowed to stand for the time ascertained as optimum for the reagent. The addition of the reagent is considerably facilitated by the use of a 5-ml. burette assembly as shown in Fig. 20.

At the end of the development time, the samples are successively balanced against the 75 μg . N per litre standard in some form of plunger-type visual colorimeter. The stronger solution is held at a cup setting of 30 mm. Under these conditions a table (Table 6) can be drawn up for

TABLE 6.—COLOUR CONVERSION TABLE (*Standard—75 μg . nitrate nitrogen per litre = 100 units.*)

Sample at 30 mm. Depth.		Standard at 30 mm. Depth.	
Balance.	Colour Units.	Balance.	Colour Units.
3	10	30	100
4	13	29	104
5	17	28	108
6	20	27	112
7	23	26	116
8	26	25	120
9	30	24	126
10	33	23	132
11	36	22	138
12	40	21	144
13	43	20	150
14	46	19	158
15	50	18	168
16	53	17	178
17	57	16	189
18	60	15	200
19	64	14	214
20	67	13	231
21	71	12	250
22	74	11	273
23	77	10	300
24	80	9	333
25	83	8	375
26	87	7	429
27	90	6	500
28	93	5	600
29	97	4	750
30	100	..	...

the conversion of balance points into colour units, at an arbitrary value of 100 units for the 75 μg . N per litre standard. By this table the appropriate number of colour units for each sample is ascertained, and its nitrate equivalence read off from the standard curve of the particular reagent in use.

At intervals of no longer than one month, preferably at fortnightly intervals, the standard curve should be checked against freshly made up nitrate standards. Any significant changes in the characteristics of the standard curve should be corrected.

(ii) Photoelectric Absorptiometer.

In Fig. 19 the results of a test of the efficiency of the blue-green filters and various absorption cells of the Spekker Absorptiometer with the June 1945*b* reagent preparation are plotted.

With the 1-cm. cell, using the blue-green and the blue filters respectively, the absorption reaches a maximum about 250 $\mu\text{g. N}$ per litre.

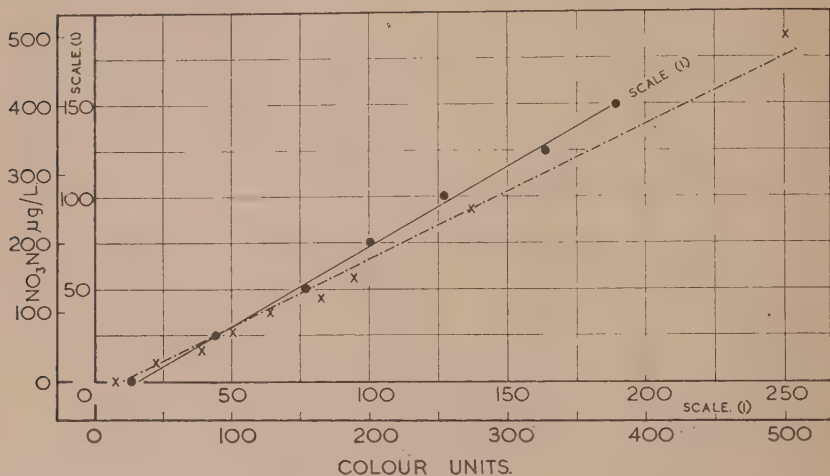


FIG. 18.—Standard curves of the August 1944 reagent.

Using 5 ml. of reagent and 5 ml. of sample, the resulting 10 ml. volume was found insufficient for use with the 4-cm. absorption cell. Therefore, at the end of the development time, 10 ml. of distilled water was added to each reaction flask and this diluted solution used in the 4-cm. absorption cell. The resulting solution displayed excellent absorption characteristics up to 500 $\mu\text{g. N}$ per litre, with the blue Ilford 602 filter.

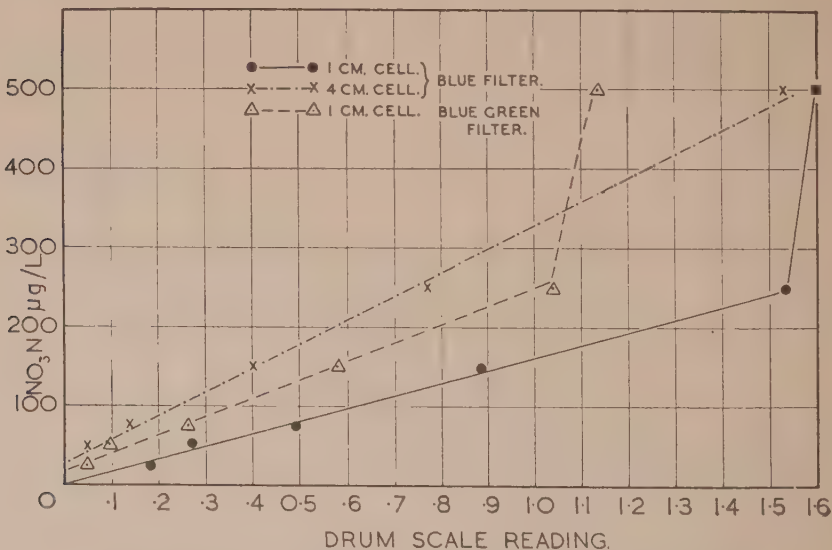


FIG. 19.—Absorption curves of the June 1945b reagent using a Spekker absorptiometer.

Similar laboratory procedure regarding addition of reagent and colour development should be followed as in Section 4 (i). At the end of the colour development, 10 ml. of distilled water is added to each flask and the absorption of these diluted standards relative to that of the distilled water blank obtained with the absorptiometer using the blue Ilford 602 filter.

A standard curve of the form given in Fig. 19 should be constructed for the reagent in use, and by reference to it any absorption setting of the drum can be converted into its nitrate nitrogen equivalence.



FIG. 20.—Photograph of delivery and developing units for the determination of nitrates.

A periodic checking of this standard curve should be made as in Section 4 (i).

5. Acknowledgments

The opportunity is taken to express the author's appreciation of the most generous assistance given by his colleague, Mr. G. L. Kesteven, in the statistical work of this paper.

His thanks are also due to Mr. G. Humphrey, of the Sydney University, and members of the C.S.I.R. Fisheries Division, for critical perusal of the draft paper.

To Misses MacGregor, Newnham, and Haynes, who have assisted in the statistical compilations, laboratory work, and preparation of text figures, respectively, the author's thanks are extended.

6. Bibliography

- Cooper, L. H. N. (1932).—The determination of nitrate in the sea by means of reduced strychnine. *J. Mar. Biol. Assoc.*, N.S., **18**: 161-6.
- Denigès, G. (1911).—Recherche rapide des nitrates et des nitrites dans l'eau à l'aide d'un nouveau réactif hydro-strychnique. *Bull. Soc. Chim. France*, **9**: 544-6.
- Harvey, H. W. (1926).—Nitrate in the sea. 1. *J. Mar. Biol. Assoc.*, N.S., **14**: 71-88.
- , (1928a).—Nitrate in the sea. 11. *Ibid.* N.S., **15**: 183-90.
- , (1928b).—Concerning methods for estimating phosphates and nitrates in solution in sea water. Section 11. Estimation of nitrates and nitrites. *Cons. Perm. Int. Explor. Mer, Rapp. Proc. Verb.*, **53**: 96.
- Riddell, W. A. (1936).—The reduced strychnine method for determining nitrates in sea water. *J. Biol. Bd. Canada*, **2**: 1-11.
- Sund, O. (1929).—The determination of nitrates in sea water. *Cons. Perm. Int. Explor. Mer, Rapp. Proc. Verb.*, **53**: 80-9.
- Thompson, T. G., and Johnson, M. W. (1930).—The sea water at the Puget Sound Biological Station from September, 1928, to September, 1929. *Publ. Puget Sound Biol. Sta.*, **7**: 345-68.
- Thompson, E. F., and Gilson, H. C. (1937).—Scientific Reports of the John Murray Expedition 1933-34, Vol. II., No. 2.
- Thomsen, H. (1937).—Dana Report, No. 12.
- Wattenberg, H. (1937).—Critical review of the methods used for determining nutrient salts and related constituents in sea water. *Cons. Perm. Int. Explor. Mer, Rapp. Proc. Verb.*, Vol. 103.
- Zwicker, B. M. G., and Robinson, R. J. (1942).—Electrolytic reduction of strychnine. Univ. of Wash. Publ. in Oceanography, Contrib. No. 116.
- Zwicker, B. M. G., and Robinson, R. J. (1944).—The photometric determination of nitrate in sea water with a strychnidine reagent. *J. Mar. Res.*, **5** (3): 214-32.

Appendix
August, 1944 Reagent.

HCl.	Dilution.	Time.	Safranin Colour Units.								
			0.	25.	50.	75.	100.	125.	150.	250.	500 μg. N/l.
		hr.									
+	×	4	4	14	30	36	52	72	98	196	360
+	×	4	6	16	34	40	64	86	120	218	340
+	×	8	6	18	34	44	..	..	110	202	378
+	×	8	4	10	16	24	..	..	50	132	330
+	×	8	6	22	38	52	..	..	120	198	312
+	×	8	6	12	20	28	..	..	62	120	312
+	×	16	4	16	36	44	..	..	94	146	264
+	×	16	4	8	14	20	..	..	42	120	202
+	×	16	6	22	48	60	..	..	106	190	258
+	×	16	6	12	20	24	..	..	52	100	224
+	×	30	10	..	58	40	52	52	..	110	132

November, 1944 Reagent.

HCl.	Dilution.	Time.	Safranin Colour Units.								
			0.	25.	50.	75.	100.	125.	150.	250.	500 μg. N/l.
		hr.									
+	×	2	6	16	..	56	64	..	94	140	260
+	×	2	6	..	..	24	30	..	42	..	104
+	×	2	8	22	..	66	74	..	104	152	304
+	×	2	8	..	..	30	40	..	60	66	124
+	×	5	8	14	..	44	120	..	156	288	440
+	×	5	6	..	..	22	72	..	88	176	332
+	×	5	10	18	..	42	58	..	78	140	310
+	×	5	10	..	..	28	42	..	50	106	156
+	×	8	8	16	..	40	..	..	46	140	200
+	×	8	8	12	..	26	..	..	52	84	120
+	×	15	8	18	..	40	..	..	70	78	88
+	×	15	6	6	..	18	..	..	32	46	86

February, 1945A Reagent.

HCl.	Dilution.	Time.	Safranin Colour Units.								
			0.	25.	50.	75.	100.	125.	150.	250.	500 μg. N/l.
		hr.									
+	×	5	2	8	..	36	54	..	82	136	180
+	×	5	2	4	..	18	24	..	50	96	154
+	×	15	4	12	..	46	66	..	92	138	198
+	×	5	4	8	..	34	38	..	76	126	198
+	×	8	2	8	..	32	50	..	68	108	144
+	×	8	2	4	..	14	24	..	36	72	108
+	×	8	4	14	..	46	66	..	86	126	144
+	×	8	4	8	—	30	42	..	62	126	137

APPENDIX.—*continued.**February, 1945_B Reagent.*

HCl.	Dilution.	Time.	Safranin Colour Units.								500 μg. N/l.
			0.	25.	50.	75.	100.	125.	150.	250.	
		hr.									
+	×	3	2	6	..	34	46	..	74	136	254
+	×	3	2	4	..	14	32	..	42	86	146
+	×	3	2	10	..	40	58	..	86	150	300
+	×	3	2	6	..	26	44	..	66	132	218
+	×	3	20	16	..	54	72	..	108	180	324
+	×	3	20	14	..	52	74	..	108	190	324
+	×	12	4	8	..	34	48	..	64	86	102
+	×	12	2	4	..	14	22	..	30	42	64
+	×	12	4	14	..	40	58	..	76	92	100
+	×	12	2	6	..	20	34	..	40	64	92
+	×	12	10	18	..	52	68	..	86	104	116
+	×	12	6	12	..	34	54	..	82	116	124

April, 1945 Reagent.

HCl.	Dilution.	Time.	Safranin Colour Units.								500 μg. N/l.
			0.	25.	50.	75.	100.	125.	150.	250.	
		hr.									
+	×	1	1	5	9	16	22	..	42	96	160
+	×	1	1	2	3	4	6	..	11	16	44
+	×	1	2	7	12	22	31	..	51	110	220
+	×	1	1	2	5	7	10	..	22	25	77
+	×	1	18	3	8	10	43	51	..	98	161
+	×	1	18	3	3	6	9	13	..	24	32
+	×	3	2	11	20	36	50	..	..	154	309
+	×	3	1	3	6	8	14	..	25	45	90
+	×	3	5	15	27	46	63	..	..	153	307
+	×	3	2	6	10	14	22	..	37	55	120
+	×	3	18	6	16	30	55	66	..	165	275
+	×	3	18	5	12	19	18	33	..	55	79
+	×	6	3	14	30	50	71	..	115	200	375
+	×	6	3	5	10	14	25	..	37	66	214
+	×	6	22	7	24	43	72	88	..	125	208
+	×	6	22	5	16	22	26	49	..	67	108
+	×	12	5	16	33	53	72	..	114	168	265
+	×	12	3	5	9	14	22	..	30	35	145
+	×	12	6	15	37	48	66	..	93	141	172
+	×	12	5	8	15	21	37	..	46	64	152

APPENDIX.—*continued.**May, 1945 Reagent.*

HCl.	Dilution.	Time.	Safranin Colour Units.								500 μg. N/l.
			0.	25.	50.	75.	100.	125.	150.	250.	
		hr.									
+	×	1 $\frac{1}{2}$	2	2	7	13	..	..	49	87	198
+	×	1 2	4	4	11	25	..	..	67	123	227
+	×	1 20	2	5	15	28	..	..	88	138	257
+	×	3 $\frac{1}{2}$	3	8	19	33	..	..	83	132	248
+	×	3 2	4	12	28	49	..	..	99	159	280
+	×	3 20	7	16	34	54	..	..	106	164	257
+	×	6 $\frac{1}{2}$	3	11	22	37	..	..	91	141	165
+	×	6 2	11	20	37	53	..	..	119	159	177
+	×	6 20	13	22	42	60	..	..	128	159	164
+	×	12 $\frac{1}{2}$	3	11	23	33	..	..	62	66	66
+	×	12 2	9	20	36	..	..	..	68	72	72
+	×	12 20	12	24	36	..	..	..	69	75	78

June, 1945 Reagent.

HCl.	Dilution.	Time.	Safranin Colour Units.								500 μg. N/l.
			0.	25.	50.	75.	100.	125.	150.	250.	
		hr.									
+	×	1 $\frac{1}{2}$	2	4	5	9	..	..	30	72	145
+	×	1 1	2	4	7	13	..	..	40	95	150
+	×	1 22	2	3	7	14	..	..	37	71	114
+	×	3 $\frac{1}{2}$	7	16	36	53	..	..	122	227	398
+	×	3 1	11	22	38	60	..	..	130	277	400
+	×	3 22	14	23	30	53	..	..	100	177	318
+	×	6 $\frac{1}{2}$	10	20	41	66	..	..	122	227	398
+	×	6 1	20	26	52	77	..	..	150	225	400
+	×	6 22	28	28	46	69	..	..	121	145	318
+	×	12 $\frac{1}{2}$	16	23	41	59	..	..	122	176	265
+	×	12 1	22	29	50	75	..	..	150	200	300
+	×	12 22	23	27	46	63	..	..	123	159	400

APPENDIX.—*continued.**December, 1945 Reagent.*

HCl.	Dilution.	Time.	Safranin Colour Units.								500 μg. N/l.	
			0.	25.	50.	75.	100.	125.	150.	250.		
		hr.										
+	×	1	$\frac{1}{2}$	2	2	2	3	..	..	6	20	60
+	×	1	$1\frac{1}{2}$	2	2	3	5	..	..	10	30	83
+	×	1	25	2	4	6	7	..	..	17	37	120
+	×	3	$\frac{1}{2}$	14	20	31	46	..	..	92	153	305
+	×	3	$1\frac{1}{2}$	15	27	43	50	..	..	100	167	333
+	×	3	25	18	28	43	56	..	..	99	187	336
+	×	6	$\frac{1}{2}$	20	27	39	55	..	..	75	153	305
+	×	6	$1\frac{1}{2}$	25	28	45	63	..	..	115	188	300
+	×	6	25	32	36	50	67	..	..	112	187	336
+	×	12	$\frac{1}{2}$	11	18	30	39	..	..	44	92	154
+	×	12	$1\frac{1}{2}$	13	22	32	45	..	..	49	94	188
+	×	12	25	19	28	37	47	..	..	53	99	168

January, 1946 Reagent.

HCl.	Dilution.	Time.	Safranin Colour Units.									
			0.	25.	50.	75.	100.	125.	150.	250.	500 μg. N/l.	
		hr.										
+	×	1	$\frac{1}{2}$	2	5	14	23	..	..	51	90	180
+	×	1	2	3	6	17	26	..	..	53	90	180
+	×	1	24	7	8	13	23	..	..	48	83	214
+	×	3	$\frac{1}{2}$	3	9	23	27	..	..	74	135	324
+	×	3	2	4	11	22	30	..	..	75	122	300
+	×	3	24	7	13	25	50	..	..	75	136	300
+	×	4.5	$\frac{1}{2}$	4	8	18	28	..	..	74	162	323
+	×	4.5	2	6	9	17	25	..	..	64	129	257
+	×	4.5	24	8	10	18	28	..	..	71	136	300
+	×	6	$\frac{1}{2}$	6	10	23	32	..	..	81	162	324
+	×	6	2	8	11	22	31	..	..	70	150	300
+	×	6	24	12	13	27	32	..	..	79	150	300
+	×	12	$\frac{1}{2}$	3	7	17	23	..	..	45	90	231
+	×	12	2	3	7	15	23	..	..	43	75	180
+	×	12	24	5	8	18	25	..	..	51	88	214

The following list gives the
BULLETINS
 numbered from 147 onwards.

A full list of the Council's publications is issued as a Supplement to the
 Council's Journal for February each year.

147. Enzootic Ataxia and Copper Deficiency of Sheep in Western Australia
148. Studies in Fertility in Sheep. 2. Seminal Changes Affecting Fertility
in Rams
149. Production of Dried Grapes in Murray Valley Irrigation Settlements.
2. Irrigation, Drainage, and Reclamation
150. The Soils of the Parishes of Longford, Cressy, and Lawrence, County
Westmorland, Tasmania
151. The Control of St. John's Wort (*Hypericum perforatum* L. var.
angustifolium D.C.) by Competing Pasture Plants
152. Soil Survey of Part of County Moira, Victoria, including the
Parishes of Boosey, Cobram, Katamatite, Naringaningalook, Katunga,
Yarroweyah, and Strathmerton
153. Pelagic Tunicates in the Plankton of South-eastern Australian Waters,
and Their Place in Oceanographic Studies
154. The Handling and Storage of Australian Oranges, Mandarins, and
Grapefruit
155. Friction and Lubrication: Report No. 2. The Lubricating Effect of Thin
Metallic Films and the Theory of the Action of Bearing Metals
156. Standardized Plant Names
157. Studies in the Biology of Australian Mullet. 1.
158. The Recovery of Inter-block Information in Quasi-Factorial Designs with
Incomplete Data. 1.
159. Poisonous and Harmful Fishes
160. The Outbreak of the Australian Plague Locust (*Chortoicetes terminifera*
Walk.) in the Season 1939-40, with Special Reference to the Influence
of Climatic Factors
161. A Review of the Evidence Concerning Expansive Reaction between
Aggregate and Cement in Concrete
162. The Soil and Land-Use Survey of the Wakool Irrigation District, New
South Wales
163. Transmission of Potato Virus Diseases. Parts 1 and 2
164. Studies in the Biology of the Skin and Fleece of Sheep. Parts 1, 2, and 3
165. Potato Virus X.: Mixtures of Strains and the Leaf Area and Yield of
Infected Potatoes
166. Fertility in Sheep. An Experimental Study of Periodicity of Oestrus
and Non-breeding Seasons in Australia
167. The Detonation of Nitroglycerine by Impact
168. A Survey, Census, and Statistical Study of the Horticultural Plantings
on the Murrumbidgee Irrigation Areas, New South Wales
169. The Entomological Control of St. John's Wort (*Hypericum perforatum*
L.) with Particular Reference to the Insect Enemies of the Weed
in Southern France
170. Pea Mosaic on *Lupinus varius* L. and other species in Western Australia
171. Experiments with Insecticides against the Red-legged Earth Mite
(*Halotydeus destructor* (Tucker)).
172. Zebu-cross cattle in Northern Australia. An Ecological Experiment
173. The Detonation of High Explosives by Impact
174. Recent Advances in the Prevention and Treatment of Blowfly Strike
in Sheep
175. The Recovery of Inter-block Information in Quasi-Factorial Designs with
Incomplete Data. 2.
176. The Analysis of Cubic Lattice Designs in Varietal Trials
177. A Soil Map of Australia
178. Food Composition Tables
179. Lubrication between the Piston Rings and Cylinder Wall of a Running
Engine
180. Studies on Deglutition in Sheep. Parts 1 and 2

BULLETINS—continued

181. Sheep Blowfly Investigations. The Attractiveness of Sheep for *Lucilia cuprina*
182. The Effectiveness of Various Mineral Dusts for the Control of Grain Pests
183. Experimental Determination of the Influence of the Red-legged Earth Mite (*Halotydeus destructor*) on a Subterranean Clover Pasture in Western Australia
184. Fellmongering Investigations. Papers I. to XII.
185. Studies on the Mitchell Grass Association in South-Western Queensland. 2.
186. The General Ecological Characteristics of the Outbreak Areas and Outbreak Years of the Australian Plague Locust (*Chortoicetes terminifera* Walk.)
187. Alcohol: Its Place in Synthetic Organic Chemical Industry
188. A Soil, Land-Use, and Erosion Survey of Part of County Victoria, South Australia, including the Hundreds of Belalie, Whyte, Reynolds, and Anne, and Part of the Hundreds of Caltowie, Yangya, and Bundaleer
189. Soils of the Berriquin Irrigation District, N.S.W.
190. Foundry Sand Resources of South Australia
191. Studies of the Physiology and Toxicology of Blowflies. Parts 10 and 11
192. Investigations of Guayule (*Parthenium argentatum* Gray) in South Australia
193. Post-Miocene Climatic and Geologic History and Its Significance in Relation to the Genesis of the Major Soil Types of South Australia
194. A Procedure of Investigation in Fisheries Biology
195. An Account of Experiments Undertaken to Determine the Natural Population Density of the Sheep Blowfly, *Lucilia cuprina* Wied.
196. Transmission of Potato Virus Diseases. Part 5
197. The Analysis of the Hydrocarbon Gases by Fractional Distillation, with Especial Reference to Cracked Tar Gases
198. Plant Responses to Molybdenum as a Fertilizer. Parts 1 and 2
199. The Use of Mineral Dusts for the Control of Wheat Pests
200. Preparation of Core Ingredients for Searchlight Carbons
201. Grazing Management: Continuous and Rotational Grazing by Merino Sheep
202. The Strain Complex and Symptom Variability of Tomato Spotted Wilt Virus
203. Agar in Australia
204. A Soil Survey of Part of Waterhouse Estate, County of Dorset, North-east Coast, Tasmania
205. Studies on the Breeding Performance of Ewes
206. Pedogenesis following the Dissection of Lateritic Regions in Southern Australia
207. The Fumigation of Wheat in Bag Stacks
208. Surface Fumigation of Insect Infestations in Bulk-Wheat Depots
209. Interaction of Insect Infestation, Temperature, and Moisture Content in Bulk-Depot Wheat
210. Preliminary Survey of the Natural Pastures of the New England District of New South Wales and a General Discussion of Their Problems
211. The Water Retting of Flax
212. The Frictional Properties of Lead-base and Tin-base Bearing Alloys
213. Laboratory and Field Tests of Mosquito Repellents
214. The Preparation and Properties of Synthetic Cryolite
215. Studies in the Biology of the Skin and Fleece of Sheep. Part 4
216. An Examination of the Peet-Grady Method for the Evaluation of Household Fly Sprays
217. The Relative Importance of Live Sheep and of Carrion as Breeding Grounds for the Australian Sheep Blowfly (*Lucilia cuprina*)
218. Studies of the Physiology and Toxicology of Blowflies. Parts 12 and 13
219. Spray Tests against Adult Mosquitoes. Parts 1 and 2
220. The Preparation and Use of Harvey's Reduced Strychnine Reagent in Oceanographical Chemistry